X-IRRADIATION–INDUCED CONGENITAL ANOMALIES IN HYBRID MICE[1]

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Heterosis, or hybrid vigor, in radiobiological studies, has been demonstrated for the adult mouse (Rugh and Wolff, 1958); for the testes (Rugh, Funk and Wohlfromm, 1961) and for some induced congenital anomalies (Rugh, Wohlfromm and Grupp, 1961). It was further shown (Grahn, 1958) that there are non-additive (heterotic) effects when a sensitive strain is crossed with a particularly resistant strain (C57BL/6 × BALB/c), while the variance in sensitivity, as measured by the dosage-mortality slope, appears to be merely additive. It was decided to carry the experiment one further generation, by crossing the hybrids with themselves as well as with each of the parental strains, in order to detect, if possible, any influence caused by the preponderance of either genotype. The test was made by x-irradiating the embryos at 8.5 days' gestation and determining the effect at 18.5 days, just prior to the expected delivery.

Materials and Method

The mice used were the CF1 Swiss white strain from Carworth Farms and the C57 BL/6 Blacks from the Bar Harbor Laboratory. These strains are easily interbred and give viable offspring. All matings were made overnight and the females with vaginal plugs were separated the following morning and marked as 0.5 day pregnant. While this exposure of the females could give a range of conception of 16 hours in simultaneously exposed mice it is now known that most of the matings occur early in this period and that all of the mice were at least 0.5 day pregnant as of 9 A.M. the morning following the introduction of males. Since the x-irradiations were to occur at 8.5 days it is believed that the time range of conception was somewhat less significant than if the exposure were shortly after conception. The number of pregnancies for any combination was at least 21 so that the time variance in fertilization is averaged out.

Whole body x-irradiation was achieved with parallel tubes in cross-fire, each at 67 cm. distance from the gravid uterus (filtration 0.28 Cu and 0.50 Al, half value layer 0.6 Cu, dose rate 50 r/min., total dose 200 r). The machine was run at 184 KVP, and 30 MA. The mice were not anesthetized, but were placed in a plastic cage within which the dosimetric determinations were made.

Since mice often destroy or eat their offspring, when they are abnormal, it was necessary in this study to sacrifice the pregnant mice at 18.5 days (prior

[1] Based in part on work performed under Contract AT (30-1)-2740 for the U. S. Atomic Energy Commission and aided by Grant RH 97 from Div. Radiological Health, Bur. State Services, Public Health Service.

to the expected delivery) and analyze the effects of x-irradiation at that time. It was also found that there was considerable variation between and within litters, even among the controls, so that to provide adequate statistical data, a minimum of 21 (and maximum of 42) pregnancies per set of categories was collected. The minimum number of implantations for any set of categories was 235 (and the maximum was 471), either figure giving a statistically adequate number so that anomaly percentages have significance. Each pregnant mouse was killed by cervical dislocation and the gravid uterus dissected out immediately. On the basis of prior studies, as well as new current findings, the categories included among the anomalous conditions were resorptions, dead fetuses, and obvious CNS, eye, visceral and tail anomalies. The "normals" included all which appeared grossly normal, but all or many of these could exhibit microscopic anomalies not readily apparent. It cannot be assumed that a "normal-appearing" fetus in a litter where there are gross anomalies caused by irradiation will itself be entirely normal. Thus, this paper categorizes only those gross anomalies which are readily identifiable, and classes as "normal" all fetuses which appear grossly to be normal.

Experimental Data

The data are presented herein by means of tables which include all of the implantations examined, namely some 296 pregnancies and 2780 implantations. It can be seen readily that 200 r at 8.5 days affects the C57 embryo much more drastically than it does the CF1 embryo of the same age, reducing the "normals" to 0.4% and 40%, respectively. It had previously been shown that the first generation hybrids were more resistant to this x-irradiation insult than were either of these parent stocks (Haverland and Gowen, 1960; Rugh, Wohlfromm and Grupp, 1961; Nash and Gowen, 1962). However, when one obtains the F_2 by mating the first hybrid generation, it is found again that there is a higher radioresistance in the appearance of more "normals" (53%). This may be suggestive of heterosis. When the first generation hybrids are crossed with either of the original parental stocks, it is found that slight heterosis is still indicated when the parent genotype is CF1 but not when it is C57. However, even in this latter cross the results are more favorable than in the C57 × C57 cross, simply because of the presence of CF1 genes, and there are as many normals as there are in the control cross of CF1 × CF1. This means simply that either the influence of the CF1 genes, in preponderance, affords some radioresistance to the embryos or when the C57 genes are in preponderance the initial first generation heterosis is reduced (see comparable data of Nash and Gowen, 1962). However, in every cross tried, the original C57 genotype, enforced by any CF1 genes, proved to be at least as radioresistant as the F_1 of the CF1 × CF1 cross. This suggests a radioresistant influence comparable to dominance.

It is known that more eggs are ovulated than are fertilized, a fact based upon the number of corpora lutea and the number of implantations (Otis, 1953). The data of this paper begin with implantations. Among these are some that are destined to die and be resorbed, in both strains of mice. But since the percentage of resorptions in the control CF1 and the control C57 mice were about the same proportion, they are included in all calculations.

The so-called "normal" mice may not, in fact, be completely normal. They

TABLE I

X-irradiation-conditioned congenital anomalies in the embryos of hybrid mice

Mouse cross		Pregnancies	Implantations	"Normal"	Dead	CNS anomalies	Anemic—stunted	Eye defects	Eviscer-ation	Resorptions	Short tails
CF1×CF1 =	C	42	471	399 (85)	4 (0.85)	0	8 (1.7)	0	0	60 (13)	0
	X	21	235	94 (40)	29 (12)	33 (14)	1 (0.43)	2 (0.85)	0	76 (32)	0
C57×C57 =	C	33	253	203 (80)	6 (2.3)	3 (1.2)	3 (1.2)	6 (2.3)	1 (0.39)	31 (12)	0
	X	34	252	1 (0.4)	18 (7.1)	0	0	1 (0.40)	1 (0.40)	231 (92)	0
C57/CF1×C57/CF1 =	C	28	269	246 (91)	0	1 (0.37)	2 (0.74)	0	0	20 (7.4)	0
	X	28	248	132 (53)	39 (16)	7 (2.8)	9 (3.6)	13 (5.2)	2 (0.8)	46 (18.6)	28 (11.3)
C57/CF1×CF1 =	C	28	273	247 (90)	3 (1.1)	1 (0.37)	0	0	0	22 (8.1)	0
	X	27	264	122 (46)	37 (14)	20 (7.6)	16 (6.1)	11 (4.1)	0	58 (22)	7 (2.6)
C57/CF1×C57 =	C	28	261	238 (91)	1 (0.4)	1 (0.4)	1 (0.4)	0	0	20 (7.7)	0
	X	27	254	102 (40)	30 (11.8)	2 (0.8)	12 (4.7)	7 (2.8)	4 (1.6)	97 (38)	12 (4.7)
Totals		296	2780								

C = Controls
X = X-irradiated to 200 r at 8.5 days gestation (Percentages in parentheses)

were normal-appearing mice since they were the survivors of x-irradiations which conditioned anomalies in the same litter, but it is very doubtful that they themselves were normal. The "dead" were those which survived until the third trimester and had the distinguishable features of fetuses. Those which were anemic and/or stunted may have been moribund, but were alive at the time of analysis. The eye defects, normally seen in C57 stock, were expressed as microphthalmia, anophthalmia, etc. In a few cases evisceration was found, in which the developing visceral organs failed to be enclosed in overgrowth of the abdomen.

Among the anomalous conditions it is important to point out that the controls are not without congenital anomalies which may well be of genetic origin (Ebert, 1961). However, the control data must be taken as the base line for comparison, in order to determine the additive effect of x-irradiation insult. Such added congenital anomalies are not genetic, but developmental. For instance, resorptions (which generally mean early embryonic death) are about the same in the two

TABLE II

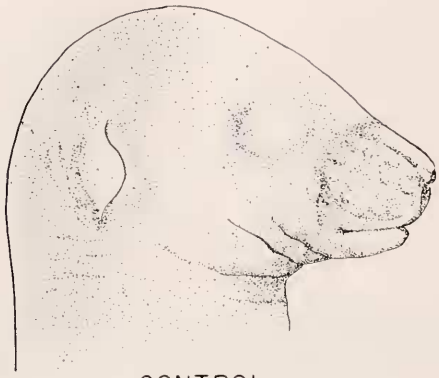
Percentage dead and anomaly risk

Group	# Implantations	# Dead & resorbed	# Survivors for CNS anomalies	# & % of CNS anomalies
CF1 × CF1 controls	471	64 (13.6%)	407	0 (0%)
CF1 × CF1 + x-rays	235	105 (44.7%)	130	33 (25.4%)
C57 × C57 controls	253	37 (14.6%)	216	3 (1.3%)
C57 × C57 + x-rays	252	249 (98.8%)	3	0 (0%)
C57/CF1 × C57/CF1 controls	269	20 (7.4%)	249	1 (0.4%)
C57/CF1 × C57/CF1 + x-rays	248	85 (34.3%)	163	7 (4.3%)
C57/CF1 × CF1 controls	273	25 (9.2%)	248	1 (0.4%)
C57/CF1 × CF1 + x-rays	264	95 (36.0%)	169	20 (11.8%)
C57/CF1 × C57 controls	261	21 (8.0%)	240	1 (0.4%)
C57/CF1 × C57 + x-rays	254	127 (50%)	127	2 (1.6%)

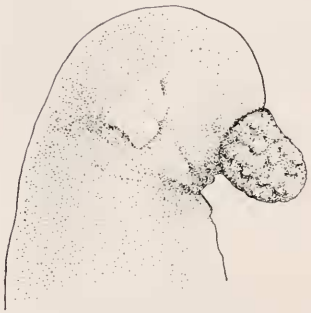
control stocks (12% and 13%) but are lower in the controls of all hybrid crosses. This would constitute added evidence of heterosis. When the embryo is x-irradiated at 8.5 days the resorption data range from 18.6% to 92%, indicating great variation in response. The highest level of resorptions occurs among the pure C57 lines (92%), indicating maximum embryonic radiosensitivity, and is lowest among the F_1 generation of the hybrid × hybrid cross. In the previous study (Rugh, Grupp and Wohlfromm, 1961) it was shown to be 90% for the C57 line. When the hybrid generation is crossed to the C57 stock, the resorptions are again high (38%) and lowest when the hybrid is crossed with the hybrid. Thus, as even among the control data, it appears that there is radioresistant heterosis when CF1 and C57 are crossed, and this heterosis is modified by outcrossings in such a way as to indicate that the presence of any CF1 genes is beneficial. When heterozygosity appears to be maximum, the conditions for surviving the radiation insult are the greatest (see Haverland and Gowen, 1960).

Consolidating the deaths, whether late fetal or early embryonic death, with resorption and considering only the survivors as having any *risk* of developing anomalies, it is obvious that the percentage of congenital anomalies of the central nervous system was always greatest when the CF1 collective influence was the greater. The resorption percentages were greatest when the C57 influences were the greater. This sort of experiment does not reveal any specific genetic influences, but rather lumps together the CF1 influences and compares them with the aggregate effect of C57 influences on the development. The anomalies are no doubt developmental, without mutational factors involved, because they arise from x-ir-

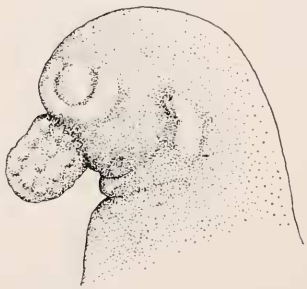
PLATE I



CONTROL 3



MICROCEPHALY
Right side 4



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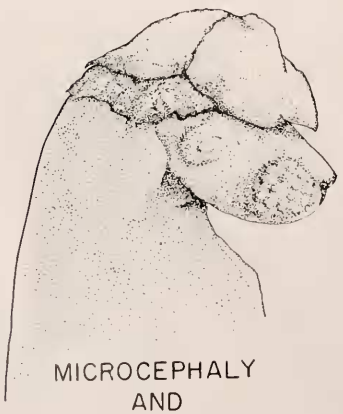
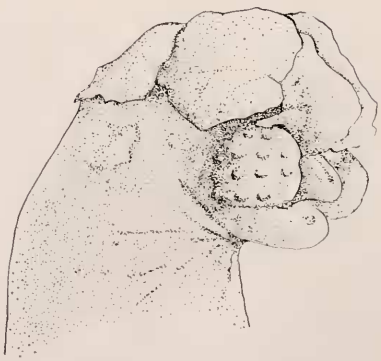


MICROCEPHALY
AND
EXENCEPHALY 6



GROSS CEPHALIC
ANOMALY 7

X-IRRADIATION-INDUCED CENTRAL NERVOUS SYSTEM ANOMALIES

PLATE II (drawings by P. Van Dyke)

radiation at almost mid-gestation. However, having indicated a genetic prepotency toward early death and resorption, in the case of the C57 mice, and toward CNS anomalies in the CF1 mice, it is necessary to suggest that this difference may be due to the fact that the basic genotype of the CF1 mice may be hardier (with regard to radiosensitivity) so that when its influences are preponderant, there is a better opportunity for CNS congenital anomalies to develop—simply because there is better survival of those with CF1 genetic factors.

Among the specific anomalies listed, those involving the central nervous system appear to be the most obvious and drastic, probably since 8.5 days represents the time of initial and most active neurogenesis. (See Plates I and II.) However, interesting data appear here in that the incidence of these congenital anomalies is more frequent among the progeny of CF1 mice than among the supposedly more radiosusceptible C57 strain. This is explained in part by the fact that the C57 strain is so radiosensitive that most of them are lost as resorptions. Among those lost were probably many that, had they survived, might have exhibited CNS anomalies. None of the few (1%) surviving x-irradiated C57 mice had gross CNS anomalies. This difference is further borne out by the fact that when the hybrid mice (CF1 × C57) are crossed with the CF1 strain more (7.6%) CNS anomalies develop (because of better survival) than when such hybrids are crossed with the C57 strain (0.8%). All x-irradiations were at 8.5 days' gestation. It is suggested that differences following the back-cross to the two strains may be related to the higher incidence of resorptions whenever the C57 genes are present, thus reducing the fetuses among which CNS anomalies could occur. Other developmental anomalies, such as stunting, anemia, evisceration, and shortened tails, did occur but not in sufficient numbers for evaluation, although the data are suggestive. These conditions cannot be of genetic origin, since the x-irradiations occurred after 8.5 days of embryonic development. They are achieved by x-irradiation interruption of the normal morphogenetic processes, aided by the subsequent deletion of necrotized 'blast cells of the actively differentiating embryo. Since eye anomalies normally occur in pure C57 stocks one would expect the C57 genic influences to be correlated with higher incidence of these defects, but this is not borne out by the data. Again this may be due to the obscuring of this tendency by the presence of CF1 genes which are not predisposed to eye defects. *The question may be asked as to whether it might be better to be so radiosensitive that there is a high death rate rather than to be less radiosensitive and survive, only to develop congenital anomalies.*

Summary and Conclusions

1. Increased radioresistance is manifested in hybrid embryos exposed to 200 r at 8.5 days of embryonic development when divergent stocks of mice are crossbred, as determined by the incidence of congenital anomalies. This is evidence of embryonic heterosis.

2. When hybrid mice are mated with hybrids (of the same crosses) or with either of the parental stocks, it is apparent that the presence of CF1 influence (genes?) affords the embryos more radioresistance only when the cross is with the CF1 line. The presence of CF1 genes brings the results up to the pure CF1 data so that it appears that there is a sort of dominance effect which becomes heterosis when the proportion of CF1 genes exceeds this minimum in these heterozygous embryos.

3. In hybrid combinations the development of CNS congenital anomalies appears to be more frequently related to the presence of CF1 genes than to those from the C57 strain. Conversely, resorptions appear to be more directly related to the presence of the C57 genes. Even in the pure strains this is substantiated because 14% of the CF1 embryos and none of the C57 embryos showed CNS anomalies following 200 r x-rays at 8.5 days' gestation. Likewise, the C57 strain embryos reacted to this exposure by producing 92% resorptions while the CF1 embryos showed only 32%.

4. The data on congenital anomalies seem at first to be confused. This is due to the simple fact of relative radioresistance of the CF1 embryos, which allows them to survive and hence to develop x-irradiation-induced congenital anomalies, particularly of the central nervous system. The radiosensitivity of the C57 strain reduced their survival. As a result of this, there are fewer survivors to develop CNS anomalies.

5. As in other studies in heterosis, it appears that this embryonic hybrid vigor is correlated with maximum heterozygosity and is reduced as this condition is diluted toward either of the parental conditions.

6. Radioresistance (or, conversely, radiosensitivity) of the embryo appears to be closely allied to inherent genetic factors quite different in the two strains of mice. In one strain there is the greater tendency to resorption and death and in the other to survival with attendant development of congenital anomalies.

LITERATURE CITED

Ebert, J. D., 1961. First International Conference in Congenital Malformations. *J. Chron. Dis.*, 13: 91–132.

Grahn, D., 1958. Acute radiation response of mice from a cross between radiosensitive and radioresistant strains. *Genetics*, 43: 835–843.

Haverland, L. H., and J. W. Gowen, 1960. Physiological vigor as a factor in radiation reduction of lifetime fertility in mice. *Rad. Res.*, 13: 356–368.

Nash, D. J., and J. W. Gowen, 1962. Effects of x-irradiation upon postnatal growth in the mouse. *Biol. Bull.*, 122: 115–136.

Otis, E., 1953. Prenatal mortality rates of seventeen radiation-induced translocations in mice. Univ. Rochester A.E.C. Pub. #291.

Rugh, R., and J. Wolff, 1958. Increased radioresistance through heterosis. *Science*, 127: 144–145.

Rugh, R., H. Funk and M. Wohlfromm, 1961. Heterosis and testes x-irradiation. *Atompraxis*, 7: 243–248.

Rugh, R., M. Wohlfromm and E. Grupp, 1961. Evidence of prenatal heterosis relating to x-ray induced congenital anomalies. *Proc. Soc. Exp. Biol. Med.*, 106: 219–221.